The stoppage of the growth of the ambulacrum and the plate abnormalities occurred approximately at the same time, and it is suggested that they may be due to functional disturbance caused by some external agent. The distortion of the test subsequent to the arrested development of the ambulacrum has been brought about by a process of regulation.

The specimen of *Echinus esculentus* above described has been deposited in the Royal Scottish Museum, Edinburgh.

EXPLANATION OF PLATE XXXIII.

Fig. 1. Test of abnormal *Echinus esculentus* viewed in plan, natural size.
Fig. 2. Test of abnormal *Echinus esculentus* viewed in elevation, natural size.

Lettering:—*m.*, madreporic plate; *t.p.*, plate which terminates Area V; the remaining symbols indicate the various areas according to Lovén's system.

3. Observations on the Minute Structure of the Spicules of Calcareous Sponges. By E. A. Minchin, M.A., V.P.Z.S., Professor of Protozoology, University of London, and D. J. Reid, M.B., C.M., F.Z.S.

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(Plates XXXIV.–XXXVII.*)

Introduction.

The minute structure of calcareous sponge-spicules has been the subject both of much laborious investigation and of many contradictory statements. In regard to the structure of siliceous sponge-spicules investigators are practically agreed upon the following points:—the mineral matter of the spicule, or spicule-ray, as the case may be, forms a hollow tube with a relatively thick wall; in the lumen of the tube, termed the axial canal, is lodged an axial filament of organic nature; the siliceous tube may be homogeneous in structure or may be stratified, that is, composed of concentric layers of silica alternating with fine layers of organic material; and the outer surface of the siliceous tube is enveloped in an outermost layer of organic substance forming a sheath to the whole spicule. Thus in siliceous spicules we find, apparently universally present, the following parts, passing from within outwards:—(1) the axial filament, a definite structure that can be isolated by hydrofluoric acid; (2) the siliceous tube, stratified or homogeneous; (3) the spicule sheath. Turning now to calcareous sponge-spicules, it is found that the only point on which all investigators are agreed is the presence of a sheath enveloping the surface of the spicule. The following brief historical summary of the statements that have been put forward will make this clear.

* For explanation of the Plates, see pp. 675–676.

Historical Review of the Question.

Grant (1826 *) pointed out that in certain sponges the skeleton is calcareous, consisting of carbonate of lime, and exhibiting no trace of phosphate of lime. In *Spongia* (*Grantia*) *compressa* he described the rays of the triradiates as "hollow within, shut at their free extremities, and having no superficial openings; but their internal cavities communicate freely at their point of junction and form there a small central reservoir." The monaxons are described as "distinctly tubular and shut at both extremities." Of the triradiates of *S.* (*Leuconia*) *nivea* he remarks, "their internal cavities are very distinctly seen."

Schmidt (1862), on the other hand, included the spicules of calcareous sponges in that category of spicules in which both central canal and lamination were entirely wanting. He considered it beyond doubt, however, that organic substance takes part in building up calcareous sponge-spicules, since heating produces small vesicles in them.

Bowerbank (1864) described the effects of heat on siliceous and calcareous sponge-spicules, and concluded that the latter contained so great a proportion of calcareous matter as to prevent their disintegration by heat. He stated the concentric stratification to be visible in the transverse fractures of any spicule, calcareous or siliceous.

Kölliker (1864), on the other hand, was unable to find any stratification or other internal structure in calcareous sponge-spicules, and considered it doubtful if they contained any organic matter, since no residue was detected if the spicules were dissolved with acids. He described the spicule-sheaths in "*Nardoa spongiosa*" (probably a synonym of *Clathrina contorta*, *vide* Minchin, 1898, p. 533, footnote, and P. Z. S. 1905, ii. p. 17). In siliceous sponge-spicules, however, Kölliker observed and described, in detail, the axial filament and the stratification of the silica.

Lieberkühn (1865) observed in the gastral rays of the quadriradiates of *Leucosolenia* "a fine layer of the contractile substance, which protrudes between the ciliated cells and either envelopes the spicule partially or completely as a fine layer, or only surrounds the foot of it as a stronger thickening (Anhäufung)." These sheaths were left behind when the spicule was dissolved with acetic acid; they were considered by him as retractile. From the description it is evident that the structures observed by Lieberkühn were really the cellular sheaths or gastral actinoblasts enveloping the projecting gastral rays, and not the true spicule-sheaths.

Carter (1869) stated that the spicules of *Grantia ciliata* differ from those of siliceous sponges in lacking a central canal; but in his later note of the same year, he modified this statement and admitted that something like a central canal may often be seen towards the base of the straight arm of a triradiate; while for the most part there is no trace of a central canal nor of the

* For bibliography, see pp. 674–5.

concentric lamination seen in siliceous spicules, although both siliceous and calcareous spicules break with a conchoidal fracture. He points out that a central canal is very obvious in siliceous spicules, "whereas in the calcareous one you can only fancy its existence here and there."

Haeckel (1872) described the structure of calcareous sponge-spicules in a most detailed manner. The spicule-sheaths were stated by him to be structureless envelopes arising as a thickness of, and separation from, the ground-substance; which, it must be remembered, Haeckel regarded as a syncytial mass of protoplasm or "sarcodine" formed by fusion of cells, and not as a secreted gelatinous mesoglœal layer, as it is now universally held to be. Haeckel contradicted Lieberkühn's statement that the sheaths were retractile. Each spicule or spicule-ray, according to Haeckel, consists of a system of numerous very thin concentric laminæ, having the form of hollow cylinders or cones, surrounding a common axis, a very fine central filament. The stratification and the axial filament were stated to be universally present, though the filament was much finer and more difficult to make out than in siliceous spicules, and sometimes not visible unless the spicule were moderately heated, whereby the axial filament was caused to turn brown and then became visible by obliquely transmitted light. The axial filament was stated to run up to the tip of the spicule and become continuous with the "sarcodine," from which it scarcely, if at all, differed in chemical nature. At the centre of triradiates and quadriradiates a small spherical hollow was to be seen, in which the axial filaments unite.

The spicules were stated by Haeckel to consist of calcium carbonate and water together with a varying amount of organic matter, termed by him "spiculin," which was left behind as a colourless and structureless residue when the spicule was dissolved by weak acids. The spiculin substance did not stain in carmine, iodine, &c., and was dissolved by caustic alkalis; its presence was stated to be best demonstrated by moderately heating the spicules. By the amount of spiculin present the spicules could be placed in a series with two extreme types, the one poor, the other rich in spiculin. The two extremes were stated to be distinguishable at first sight under the microscope, the spicules rich in spiculin appearing darker, more refractile than those poor in spiculin, which were dull and pale in appearance. The phylogenetically older forms of spicules, that is to say, the monaxons of simple form and the regular triradiates, were stated to contain least spiculin; the phylogenetically younger forms of spicules, such as the sagittal triradiates, possessed most spiculin.

Lendenfeld (1885) stated that spicules of calcareous sponges consisted of carbonate of lime mixed with organic substance; by treatment with gold-potassium chloride the spicule was shown to consist of "a great number of small prisms, parallel to one another, radiating from the axis," which was "a cylindrical cord of organic matter without lime." The oldest part of the spicule

was stated to contain more organic substance than the younger, *i. e.* outer parts.

An entirely new epoch in the study of calcareous sponge-spicules was inaugurated by the elaborate and exhaustive investigations of Ebner (1887), by whom and by Sollas, independently, the peculiar crystalline nature of these spicules was discovered; namely, the fact that each spicule, whatever its form, behaves optically like a single crystal of calcite. As regards the minute structure of the calcareous spicules, Ebner's results differ totally from those of Haeckel. Ebner was unable to find any residue after dissolving the spicules with various acids, and his attempts to demonstrate any such organic residue with stains gave negative results. He explained the optical differences between the two types of spicules, described by Haeckel as rich and poor in spiculin respectively, by the fact that in the regular triradiates the crystalline optic axis is vertical while in sagittal forms it is inclined or even horizontal, in the facial aspect of the spicules. Ebner pointed out further that the "browning" of the spicules produced by heating is not due to the formation of carbon through charring of organic substance, but is due to the disengagement of fine bubbles of gas in the substance of the spicule (compare Schmidt), making it opaque by transmitted light, milk-white by reflected light; with stronger heating the gas breaks up the spicule with decrepitation. Ebner found, however, certain differences between calcareous sponge-spicules and pure calcite, and analysis showed the presence of magnesium, sodium, and sulphates, as impurities mixed with the calcite comprising the spicules.

According to Ebner the axial filament of Haeckel is due to the axial portion of the spicule having a different composition to the peripheral portion, rendering the axis more easily attacked by acids, by the action of heat, &c.; but the difference between axis and periphery is a gradual and quantitative, not a sharp qualitative contrast. Ebner found the alleged stratification to be present only in a few spicules, mostly very large forms. He studied the stratification of the huge triradiates of *Leucaltis solida*, and the large monaxons of *Leucandra aspera* and *L. alcicornis*, and found it also due to a special distribution of more, or less, decomposable substances in different parts of the spicule. Thus the appearance of an axial filament and of stratification are both due to a similar cause, namely, the periodic deposition of more, or less, pure calcite in the building up of the spicule.

Ebner concluded from his observations that the spicules of Calcarea are mixed crystals consisting chiefly of calcite without organic substance, but containing inorganic impurities (Na, Mg, S, probably also water), and that the conditions of the mixture differ at different periods of the growth and in different part sof the spicule.

Lendenfeld (1891), while quoting Ebner's results, reiterated his former statements to the effect that "Each spicule-ray

consists of a thin, somewhat turbid (*trüb*) axial thread, enveloped by the hyaline (*glashell*) spicule-substance. In the latter a concentric stratification round the axial filament is to be recognised" (*l. c.* p. 369).

Minchin (1898, p. 569) stated that the spicules of *Clathrina coriacea*, if treated with picro-nigrosin (saturated solution of picric acid in water, 9 volumes; 1 % nigrosin in water 1 vol.), showed the sheath and the axial filament, left behind after solution of the calcareous matter, and stained blue by the nigrosin.

Bütschli (1901) published elaborate investigations upon the minute structure of siliceous and calcareous sponge-spicules, taking the large monaxons of *Leucandra aspera* as examples of calcareous spicules. His results were, in the main, confirmatory of Ebner's. He found that moderate heating produced a finely alveolar structure in the spicule. No axial canal or filament was found to be present; the axial thread, sometimes visible after moderate heating, was stated to be due to a modification of the calcareous substance, which at the central part of the spicule was distinguished from the remaining part by greater refractility and by being more easily attacked by acids. No trace of an axial filament or sheath was found when spicules were dissolved by acetic acid. The stratification was ascribed to the concentric arrangement of the minute alveoli composing the spicule.

Unlike Ebner, Bütschli found small quantities of organic substance left when the spicules were dissolved with hydrochloric acid. The spicule-sheaths were found to be not purely organic in nature but to contain a certain amount of inorganic matter, probably calcium carbonate.

Maas (1904) and Weinschenk (1905) agree in assuming that the calcite of the spicule must be combined with finely divided organic substance. Maas explains the solvent action of caustic alkalis on the spicules by the supposition that the caustic attacks a substance holding together the constituent particles, and so produces a disaggregation of calcareous elements which were formerly crystallographically orientated. He considers the phenomena seen on heating to be explicable also on the assumption of a finely distributed organic material in the spicule; and he showed that when the sponge is grown in water deprived of $CaCo_3$ the organic substratum alone of the spicule is secreted. Weinschenk dwells on the differences between calcareous sponge-spicules and pure calcite, and considers these differences, and also the peculiar form of the spicules, explicable only by the presence of a fine organic tissue in the spicules.

Bütschli (1906) controverts the statements of Weinschenk upon certain points which are of secondary importance for the subject of this memoir, and maintains his former position. He denies that the opacity and decrepitation produced by heating is due to the presence of organic matter in the spicules.

It is seen from the foregoing that opinions are greatly divided upon the question of the structure and composition of calcareous

sponge-spicules, and especially with respect to the amount of organic matter present in them. While Haeckel, Lendenfeld, Maas, and Weinschenk assert or assume the presence of considerable quantities of organic substance in the spicules, Kölliker and Ebner allow none at all except in the sheath, and Bütschli admits the existence only of a trace of organic matter and considers even the sheath to be largely inorganic. It should be further pointed out that the two most detailed investigations upon this question, namely, those of Ebner and Bütschli, were based, so far as the composition of the spicules is concerned, on a very limited number of forms; Bütschli, in fact, studied only the large monaxons of *Leucandra aspera*. Hence there is a possibility that their investigations do not cover the whole range of variation that those spicules may present. Both Ebner and Bütschli worked at the largest types of spicule that they could obtain, in order to facilitate the handling and treatment of the material.

Observations upon Calcareous Sponge-spicules.

Our investigations have been directed towards endeavouring to demonstrate the existence of a residue after decalcification, by means of specific stains, as Ebner attempted to do, but without success. It is obvious that if any organic residue were left after decalcification, it might be expected to have an affinity for certain stains and not for others, and might therefore be demonstrable only by means of particular dyes, so that the negative results obtained by Ebner would not necessarily disprove the existence of an organic residue. We obtained in all cases positive results with nigrosin, as stated previously by one of us (Minchin, 1898), and also with the allied stain indulin. In our investigation we have made use chiefly of the spicules of *Clathrina contorta*, but we have examined the spicules of several other species: of Clathrinidæ, *Clathrina clathrus* and *Ascandra falcata*; of Leucosoleniidæ, *Leucosolenia lieberkühnii* and *L. complicata*; and of Heterocœla, *Sycon ciliatum*, *Leucandra aspera*, and *Heteropegma nodus-gordii*.

Our method of procedure was as follows. A piece of the sponge taken from a specimen preserved in alcohol was washed in water and placed in a tube of a small hand-centrifuge with a few drops of Eau-de-Javelle, and gently shaken. In a short time, generally about half-a-minute, the sponge is dissolved into a cloud of spicules. The tube is then filled up with distilled water and shaken up, and then with the centrifuge the spicules are driven down to the bottom of the tube. The liquid is then carefully poured off, taking care not to disturb the spicules, the tube is filled up again with water, shaken up, and the process repeated. In this way the spicules can be given three or four washings in as many minutes, and are freed both from organic matter of the sponge-body and from the Eau-de-Javelle. The next procedure was usually to add to the tube containing the

spicules in distilled water a few drops of ordinary glycerine and albumen-solution, such as is used for sticking sections on slides. The spicules were shaken up in this and then centrifuged down, after which a drop or two of the fluid, with the spicules, was drawn up with a pipette, spread out on a slide, and dried off on the paraffin oven. When dry, the spicules were fixed on the slide by plunging it into absolute alcohol, whereby the glycerine is extracted and the albumen coagulated. The spicules can now be decalcified and stained in any way that is desired. Other methods of imbedding and fixing the spicules were also tried but were not satisfactory. By means of the albumen solution, provided that neither too much nor too little be used, good permanent preparations of the decalcified and stained spicules can be made and mounted in Canada balsam.

In addition to this method, spicules in distilled water, without addition of albumen, were treated with acids and stains and the effects of them watched under the microscope. Since the spicules treated in this manner were not fixed to the slide, it was impossible to wash out the stain and mount them permanently in Canada balsam, but it was possible to observe in detail the effects of the acids and stains upon the spicules, and there was the advantage that the results were not complicated by the presence of the albumen, which is itself stained by both nigrosin and indulin.

The Spicules of Clathrina contorta *.

We shall begin with an account of the results attained with the spicules of *Clathrina contorta*, of which we had a very abundant material; the differences presented by other species will be noted subsequently. Spicules fixed to the slide with albumen were treated for about half-an-hour with a combination of an acid and a stain in the following proportions:—

$\frac{1}{2}$% to 1% of acid in distilled water, except in the case of picric acid of which a saturated solution was used	9 vols.
1% stain in distilled water	1 vol.

In this way, picric, nitric, acetic, and hydrochloric acids were combined, respectively, with either nigrosin or indulin as a stain. After staining for 20 minutes or half-an-hour, the preparations were washed with distilled water, absolute alcohol, oil of cloves, and mounted in Canada balsam. The results in all cases were the same. The spicule was completely decalcified, and left behind a deeply stained sheath, and an axial filament in each ray (figs. 1–10). The best and clearest results were obtained with the picric acid combinations; with the other acids the filaments were stained just as deeply, but there was a frequent tendency to form a flaky deposit which obscured the result.

* For an account of the spiculation and nomenclature of this sponge, see Minchin, P. Z. S. 1905, ii. pp. 3–20.

The same combinations of acids and stains were also applied to the spicules placed on the slide in water, without any albumen. When treated in this way, the sheath of the spicule stains so deeply as to largely obscure the axial filament, either on account of the spicule being exposed on all sides to the action of the stain, or because the preparation does not go through the processes of washing and clearing necessary for a permanent preparation, processes which probably extract a certain amount of the colour. The deep colour of the sheath is especially marked in the combinations with nitric, hydrochloric, and acetic acids, more so than with picric acid. The fact that the sheath stains so intensely is of interest, since it shows that the action of Eau-de-Javelle in isolating the sponge-spicules does not destroy the sheath. When the spicules are fixed to the slide with albumen, each spicule appears after treatment with the combined acid and stain as a space or mould in the layer of albumen limited by a deeply-stained contour (compare fig. 1, Pl. XXXIV., fig. 5, Pl. XXXV., and fig. 8, Pl. XXXVII. especially); but since the albumen takes the stain also, the sheath cannot be distinguished with certainty as a structure separate from the enveloping albumen, in spicules fixed in this way. The fact that the spicule-sheath is not dissolved in Eau-de-Javelle is in favour of Bütschli's view that the sheath is chiefly inorganic in nature; a conclusion founded by him on the observation that the sheath could be isolated by means of caustic potash (35%), though this reagent dissolved the rest of the spicule, and that sheaths so isolated were dissolved by strong acetic acid.

When the processes of decalcification and staining were watched under the microscope, it was observed that the picric acid combinations did not break up the spicules so much as the other acids, even when these were used in strengths much lower than those quoted above. With picric acid and nigrosin combined, the filament appears first at the tip of the ray, and as the decalcification goes on, the filament appears as if traced by the tip of the gradually receding calcite, until it reaches the centre, when decalcification is complete. With hydrochloric and nitric acid combinations the decalcification does not go on so regularly; fragments of the spicules are frequently seen to be cut off from the rest of the spicule, and when separated, the fragments rush along to the tips of the rays as if impelled by powerful currents. The violence of the action of the acid was most marked with hydrochloric, less with nitric, and least with acetic acid; it probably accounts for the fact that the axial filaments are not, as a rule, so well shown with these acids as with the picric-acid combinations.

In addition to the combinations of acids and stains mentioned above, many experiments were made with acids and stains used separately. When clean spicules, placed in distilled water on the slide without any albumen, were treated with acids, it was usually observed, especially when acetic acid was used, that the whole spicule seemed to disappear, leaving only the axial thread, without

any sheath. Bütschli also (1908, p. 317) was unable to find any remains of the sheath after dissolving the spicules with acid. When, however, the acid was combined with the stain, both filament and sheath were left intact and stained. Hence it is probable that the disappearance of the sheath, when acid alone is used, is due, not to the destruction of the sheath by acid, but to its collapsing on the filament. This conclusion is supported by the fact that when spicules stuck on with albumen are treated with a combination of acetic acid and nigrosin, many of them appear to contain unusually thick filaments, which are seen on closer inspection to consist of the true axial filament together with the collapsed sheath. Acetic acid would thus seem to have a solvent or partially softening action upon the sheath. Bütschli also found (1906, p. 317) that spicule-sheaths isolated by caustic potash were dissolved completely by strong acetic acid, but were preserved by very dilute acetic. Attempts to decalcify the spicules with acid first and then to stain the sheath and axial filament subsequently with nigrosin or indulin, were successful when picric, nitric, or acetic acid were used, but not with hydrochloric acid. Various other stains were used without any effect on the filament, for example carmine stains (borax- and alum-carmine), Kernschwarz, indigo-carmine, &c.

Appearance and Structure of the Axial Filament.

The axial filament occupies a central position in the axis of the spicule-ray. By focussing carefully the upper and lower surfaces of the spicule-sheath in a spicule, decalcified and stained, it can be clearly seen that the filament lies midway between the two surfaces. In the optical transverse section of a ray, such as can be easily obtained in the case of the gastral rays of the quadriradiates (fig. 4, Pl. XXXV.), the filament appears as a black dot occupying the centre of the ray, and can be traced up and down the ray by focussing. The axial filament exhibits a certain amount of tenacity and strength: this is shown by the fact that when the decalcification proceeds irregularly in a spicule imbedded in albumen, detached fragments of calcite may be held still for a time by the filament, until set free either by the filament giving way under the strain, or by decalcification taking place at the centre of the fragment round the filament, after which the loose fragment rushes along inside the sheath of the spicule. In spicules not imbedded in albumen, the sheath sometimes breaks across the ray, after decalcification and staining, but the distal part of the sheath is held on by the filament, which stands a great deal of bending and washing about without breaking across. In such preparations it is clearly seen that the sheath is a very delicate structure, much less strong and resistant than the actual filament.

In the triradiate systems the axial filament appears to start from the extreme tip of each ray of the spicule, and to be con-

tinuous at this point with the sheath (fig. 8, Pl. XXXVII.). The terminal portions of the filaments are very slender and delicate, but they soon become thicker as we pass towards the centre of the spicule, and in the greater part of the shaft of each ray the axial filament is a coarse structure very obvious when stained. At the junction of the rays the filament widens out very greatly and forms a cobweb-like arrangement, usually of triangular shape, which may be termed the central triangle, and occupies the centre of the triradiate system (figs. 2, 5–7, Pl. XXXV.). By comparing different spicules, it is seen that the structure of the central part varies. In those spicules which have developed a fourth ray and become quadriradiates, each of the three axial filaments of the triradiate system is continued into the central triangle, but usually not quite to the central point of the spicule: the filament seems to break up as it were, to form the triangle (figs. 6, 7). On the other hand, in the triradiates with no gastral ray, the axial filaments are continued scarcely diminished to the centre and there become continuous, and the central triangle is very faint (fig. 2). This arrangement, though specially characteristic of the triradiates, is sometimes seen also in the quadriradiates (fig. 5).

The monaxons of *C. contorta* were found very difficult to deal with on account of their huge size. In the albuminised preparations they are only partly covered by the albumen, hence the sheath stains very deeply, just as in the triradiates when they are stained without being imbedded. Further, when they are cleared and mounted in Canada balsam after staining, the larger monaxons collapse. Smaller monaxons, however, give satisfactory preparations from which good photographs can be taken (figs. 9, 10, Pl. XXXV.). It is seen that the monaxons contain an axial filament which commences at each extremity of the spicule as a fine thread, and as it passes towards the middle point of the spicule the thread widens out so as to be represented by a double contoured band, which extends through the greater part of the shaft of the monaxon. In some monaxons the band may be quite one-third the width of the spicule; in others it is comparatively narrower.

The monaxons of *C. contorta* require decalcification for not less than half-an-hour, or even longer.

The Spicules of other Calcareous Sponges.

In the Clathrinidæ examined, namely *C. clathrus* and *Ascandra falcata*, we have found the filaments exceedingly distinct, as was noted by Minchin (1898), in *C. coriacea*, when stained by the methods above described; and there is scarcely any difference to be noted except in minor points, from what has been described in *Clathrina contorta*. *Ascandra falcata* is a very favourable object for studying the filaments, especially in the characteristic sickle-shaped monaxons (fig. 12, Pl. XXXIV., and figs. 13, 14,

Pl. XXXVII.) which are abundant and easily found, and at the same time are not so inconveniently large as in *C. contorta*. It is seen that in the distal blunt curved portion (fig. 13) the axial filament is very thick, and forms a broad band showing a dark double contour enclosing a central lighter portion; in the proximal straight pointed portion (fig. 14) the filament appears as a single thread, as in the rays of the triradiates. Hence the monaxons of *A. falcata* show a noteworthy difference from those of *C. contorta*, a point to which we shall return.

In *C. clathrus* (fig. 15, Pl. XXXVI., fig. 16, Pl. XXXIV., and fig. 17, Pl. XXXVII.) it was found that in the majority of the triradiates the axial filament terminated abruptly at an appreciable distance from the end of the spicule, and was not continuous with the sheath. This condition is probably correlated with a peculiarity in the mode of growth of the spicules of this species which was pointed out by Minchin (1898), namely, that the apical formative cell or "founder" does not leave the ray, but persists and helps, apparently, to secrete the blunt thickened termination of the ray which characterises this species. In a few cases, however, a continuation of the axial filament up to the sheath could be seen distinctly (fig. 16), but from the shape of the rays it is probable that in such cases the spicules were not quite full-grown.

The spicules of Leucosoleniidæ and Heterocœla examined by us appear, with one exception presently to be described, very different from those of Clathrinidæ. The first impression derived from examination of them is that no axial filament is present. A more careful study reveals a filament presenting a certain similarity to that of the monaxons of *Ascandra falcata*, namely, a broad band towards the base of each ray, which narrows to a delicate filament towards the tip of the ray (fig. 18, Pl. XXXVII.). It is very difficult to get satisfactory photographs of the filament, both on account of its feeble staining powers, and of the difficulty of getting it in focus, due to the frequent curvature of the rays. In connection with the phylogenetic speculations of Minchin (1900, p. 109, and 1908), it is of interest to find so great a difference in the structure of the spicules of Clathrinidæ on the one hand, and of Leucosoleniidæ and Heterocœla on the other hand. It may be pointed out further that, as stated above, the studies of Ebner and Bütschli, with regard to the presence of an axial filament, were based entirely upon examples of the Heterocœla.

An exception, however, to the foregoing statements concerning Heterocœla is furnished by the remarkable sponge *Heteropegma nodus-gordii* Polej., of which, by the kindness of Professor Dendy, we have been able to examine a specimen. In this sponge we find the axial filaments very distinct, especially in the triradiates of moderate size *, in which they present the same characters as in *Clathrina contorta*, and can be photographed easily at low

* For figures of the spiculation of *Heteropegma* see Poléjaeff, 'Challenger' Reports, Zool. vol. viii. part xxiv. (1883) pl. iv. figs. 1, *a–d*.

magnifications. The very large triradiates, however, tend to collapse when decalcified, like the monaxons of *C. contorta*, so that it is difficult to obtain satisfactory preparations of them. In the minute triradiates also the filaments are clearly seen, and present no other difficulties to the photographer than such as are caused by their minute size, and by the fact that the rays usually lie in different planes. A study of the filaments in this sponge brings out a point of some morphological interest. The minute triradiates, as is well known, are of two types of form, regular and sagittal. An examination of the filaments shows, however, that in both forms alike the filaments meet at the centre at angles of 120° (fig. 22, Pl. XXXIV., fig. 23, Pl. XXXVI.)*. Thus the spicules of *Heteropegma* are distinctly Clathrinid in type, and the sagittal forms occurring in this sponge are to be regarded as arising simply by secondary curvature of the rays of a primitively regular triradiate; they may be termed pseudo-sagittal. It has already been pointed out by Bidder (1898) and Minchin (1900, p. 109), that *Heteropegma* is a sponge which stands apart from other Heterocœla and approaches the Clathrinidæ in its characters; and the study of its axial filaments certainly supports these conclusions.

The Nature of the Axial Filament.

If we compare one of the photographs given here (figs. 2, 6–8) of the axial filaments of the triradiate systems of *Clathrina contorta*, magnified 1000 diameters, with the figure of a young spicule of this sponge given by Minchin (1898, pl. 42. fig. 49) at the same magnification, we are at once struck by the fact that the central triangle, as we have termed it, formed by the axial filaments, is nearly as large as the whole spicule at this early stage; much larger, in fact, than the earliest stages of the spicules that can be found.

It was further shown by Minchin (*l. c.* pp. 572–579) that the spicules in early stages of development, when examined between crossed nicols, light up scarcely or not at all. Hence in early stages the spicules must contain very little, if any, crystalline substance, that is to say calcite, but must consist chiefly of non-crystalline substances, perhaps both organic and inorganic. We have referred above to Ebner's statements as regards the inorganic impurities in the spicule. It must be supposed that the first portions of the spicule formed consist chiefly of these "impurities," and that the axis of the spicule, as it grows in length, is also formed of substance containing very little calcite. On the other hand, the portion of the spicule formed later is

* In all the preparations the axial filaments are very liable to become displaced, since they are entirely unsupported after decalcification of the spicule. In the spicule photographed in fig. 23, it can be seen that the filament of the left-hand ray is displaced, but that of the right-hand ray shows the typical regular angle.

almost pure calcite; but the layer formed last of all, namely the sheath, is again an "impure" layer.

Having regard to the mode of formation of these spicules discovered by Minchin (1898 and 1908) and Woodland (1905), it would be a tempting hypothesis to refer the two substances secreted to the activities of the two formative cells; the apical formative cell or "founder" may be supposed to lay down the "impure" substance, while the basal formative cell or "thickener" secretes the purest calcite. On the other hand, the formation of the sheath must also be ascribed to the thickener.

The continuity, generally to be observed, of axial filament and sheath, and the similarity of their staining reactions are points in favour of considering these two structures to be of similar nature. We have referred above to Bütschli's arguments in favour of regarding the sheath as being chiefly of inorganic nature, a conclusion for which there is much to be said, and which may be extended to the axial filament. The fact, however, that both filament and sheath have an affinity for special stains, is in favour of their containing a certain amount of organic matter, and we may regard sheath and filament as consisting of an organic basis richly impregnated with inorganic non-crystalline materials. At this point we must leave the question of the nature of these structures to receive more exact and definite solution from more competent observers. We claim merely to have demonstrated the following proposition:—*The spicules of calcareous sponges leave after decalcification a residue in the form of structural constituents, sheath and axial filament, which can be coloured by special stains.*

In conclusion, attention may be drawn to some points relating to the morphology of the spicules, upon which the axial filaments throw some light. It is seen that in the rays of the triradiates, the filament is broad and even band-like at the base, and tapers to a fine point at the apex. Comparing with this the monaxon of *A. falcata* (fig. 12, Pl. XXXIV., figs. 13, 14, Pl. XXXVII.), it is seen that the filament is broad and band-like at the blunt distal end of the spicule, and tapers to a fine thread at the pointed proximal end. This supports the conclusion, based by Minchin (1908) upon developmental data, that the distal projecting ends of the monaxons are homologous with the central ends of the rays of the triradiates.

Comparing, however, the monaxons of *C. contorta* with those of *A. falcata* (figs. 9, 10, Pl. XXXV.), it is seen that in *C. contorta* the filament is band-like towards the middle of the spicule, but tapers to a fine thread at each end. This strongly suggests that the monaxons of this sponge are not really primary monaxons, but are secondarily derived from triradiates and are to be regarded as biradiates as suggested by Minchin (P. Z. S. 1905, ii. p. 10). On the other hand, the monaxons of *A. falcata* would appear to be true primary monaxons.

Addendum.

Intracellular Networks in the Gastral Layer.

A point of some interest was observed in a preparation made in the following manner. A piece of the body-wall of *Clathrina contorta* (preserved in alcohol) was stained with picro-nigrosin, and passed through water and alcohols into oil of cloves; then the inner gastral surface was brushed gently with a soft paint-brush to remove the gastral layer of collared epithelium; finally, the piece was mounted in Canada balsam with the gastral surface uppermost. The upper surface of the preparation then showed a delicate honeycomb-like network, stained blue with the nigrosin, enclosing irregular polygonal meshes fairly uniform in size, with here and there a much larger rounded mesh and occasionally a small, circular mesh. As the preparation was not all in one plane, only small stretches of the network could be sharply photographed (fig. 24, Pl. XXXVII.). The polygonal meshes represent spaces formerly occupied by collar-cells, many of which are to be seen still *in situ* in the preparation; the large rounded meshes are spaces left by porocytes; and the small circular meshes are shown by their relations to underlying triradiate systems to be the spaces occupied by the gastral rays of quadriradiates. The network itself is an extension of the gelatinous ground-substance between the bases of the collar-cells and gastral rays and round the inner ends of the porocytes, probably forming a cementing substance, as it were, helping to keep the easily detached collar-cells in their places.

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EXPLANATION OF THE PLATES.

All the photographs are from spicules stuck on the slide with albumen, decalcified and stained with picro-nigrosin, and mounted in Canada balsam.

Figs. 1–10. *Clathrina contorta.*

Fig. 1, Pl. XXXIV. A triradiate. × 400.
2, Pl. XXXV. The central portion of another triradiate. × 1000.
3, Pl. XXXIV. A quadriradiate, gastral aspect. × 400.
4, Pl. XXXV. A quadriradiate, gastral aspect, at a high focus to show the gastral ray in optical transverse section. × 1000.
5, Pl. XXXV. The central part of a quadriradiate. × 1000.
6, Pl. XXXV. The central part of another quadriradiate. × 1000.
7, Pl. XXXV. The central part of another quadriradiate. × 1000.
8, Pl. XXXVII. The extremity of a ray of a quadriradiate. × 1000.
9, Pl. XXXV. A small-sized monaxon. × 150.
10, Pl. XXXV. Another small monaxon. × 150.

Figs. 11–14. *Ascandra falcata.*

Fig. 11, Pl. XXXIV. Central portion of a triradiate. × 1000.
12, Pl. XXXIV. A monaxon. × 250.
13, Pl. XXXVII. Distal extremity of a monaxon. × 500.
14, Pl. XXXVII. Proximal extremity of a monaxon. ×

Figs. 15–17. *Clathrina clathrus.*

Fig. 15, Pl. XXXVI. The extremities of two triradiates and a broken ray of a third. × 1000.
16, Pl. XXXIV. The extremity of a triradiate. × 1000.
17, Pl. XXXVII. The central part of a triradiate. × 1000.

Figs. 18, 19. *Leucandra aspera.*

Fig. 18, Pl. XXXVII. A triradiate showing the double-contoured filaments. × 250.
19, Pl. XXXVI. A quadriradiate. × 500.

Figs. 20, 21. *Sycon ciliatum.*

Fig. 20, Pl. XXXVI. A triradiate. × 500.
21, Pl. XXXVI. The same triradiate at a slightly lower focus. × 500.

Figs. 22, 23. *Heteropegma nodus-gordii.*

Fig. 22, Pl. XXXIV. A small triradiate (one ray broken). × 1000.
23, Pl. XXXVI. A small sagittal triradiate, showing the filaments; on the left the filament has become displaced. × 1000.

Fig. 24, Pl. XXXVII. Photograph of the gastral surface of the body-wall of *Clathrina contorta*, stained with picro-nigrosin, the collar-cells brushed off; showing the network left between the collar-cells, porocytes, and gastral rays. Owing to this network not being exactly in one plane, it is not seen all over the photograph. × 1000.

4. Two New Genera (and a New Species) of Indian Lycænids. By T. A. Chapman, M.D., F.Z.S.

[Received May 14, 1908.]

(Plate XXXVIII.*)

In trying to gain some knowledge of the genus *Cyaniris* by examining the ancillary appendages, I met with much trouble over *Cyaniris chennellii* de Nicév. I obtained specimens from various sources, and informed various people that they had a *Zizera* or something thereabouts, and not a *Cyaniris*. Herein I was right, but so were they, their insect being *chennellii* de Nicév. I stuck to my guns unnecessarily, largely because Col. Bingham found in his collection a specimen that was certainly not a *Zizera* but probably a *Cyaniris*, and which he had compared with the type of *chennellii* and found to agree. I took it therefore that this was *chennellii*, but could come across no other specimen. I also, of course, assumed de Nicéville to know what was and what was not a *Cyaniris*, and that he would not call a *Zizera*-like species a *Cyaniris*. It turns out, however, that this was precisely what he did do, and in doing which, succeeding authorities appear to have

* For explanation of the Plate see p. 678.